



Full Length Article

Effect of Supplementation of Chinese Herbal Extracts in Drinking Water on Growth Performance, Nutrient Utilization and Immune Response in Broilers

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Abstract

The objective of current trial was to evaluate the effect of three Chinese herbal extracts (*Rhubarb*, *Coptis chinensis* and *Codonopsis*) supplementation in drinking water as alternative to antibiotics on performance and immune organs weight/index in growing broilers chicks. For this purpose, 300 days old broiler chicks were reared in floor pens and raised on commercial feed for 7 days for adaptation. After seven days, birds were distributed into five groups in such a way that each group contained 6 replicates and each replicate had 10 birds. All groups were fed commercial diet and offered water with or without additives. In control group drinking water was without any additives; In second group drinking water was supplemented with antibiotic (Flavomycin 10 mL/liter). The remaining three groups were supplemented with *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract @ 20 mL/liter, 15 mL/liter, and 10 mL/liter in drinking water, respectively. Results indicated improved growth performance in broiler had supplementation of Chinese herbal extracts in drinking water as compared to control ($P < 0.05$). A significant decrease in cholesterol and blood glucose was observed in those birds which had supplementation of Chinese herbs extracts in drinking water ($P < 0.05$). Moreover, Chinese herbs extracts in drinking water improved immune organ weight and immune indexes in broiler. It could be concluded that supplementation Chinese herbs extracts in drinking water had positive influence on growth performance, blood biochemistry and immune organs in broiler. © 2019 Friends Science Publishers

Key words: *Rhubarb*; *Coptis chinensis*; *Codonopsis*; Growth performance; Broiler

Introduction

Phytogenics, pharmaceutical plants, and herbs are being used in human food for different purpose (Saleh *et al.*, 2018). In recent years, the use of phytogenics, pharmaceutical plants, and herbs has been increased in the diet of poultry as fed additive to boost health and comfort of birds (Panda *et al.*, 2006; Gong *et al.*, 2014; Mushtaq *et al.*, 2016). The use of herbs or herbs extract as alternative growth promotor has been increased due to ban of antibiotics as growth promotor in poultry diet (Mushtaq *et al.*, 2016; Omar *et al.*, 2016). In commercial diet of poultry different types of antibiotics are used to reduce load of harmful microorganisms in intestine of the bird and to improve growth performance, feed conversion ratio, and immunity (Panda *et al.*, 2006; Seyed *et al.*, 2013). However, different countries have banned antibiotics use in animal diets due to efflorescence of microbe resistance (Leeson 2007; Steiner 2009). Therefore, scope of use of herb or herb extract is increasing as growth promotor.

In livestock nutrition generally and poultry nutrition specifically, a large number of herbs has been used to

evaluate herbs potential as growth promotor, coccidiostatic, antimicrobial, anthelmintic, and immune-stimulating (Panda *et al.*, 2006). Among different herbs, Chinese herbs are the most popular options as a substitute of antibiotic growth promoters because Chinese herbs have been proved to have a great effect toward immune response, intestinal health, nutrient metabolism and growth in animals (Gong *et al.*, 2014). However, studies of Chinese herbs like *Rheum rhabarbarum* (*Rhubarb*), *Coptis chinensis*, and *Codonopsis* in animal feed generally and poultry feed specifically are limited.

Rhubarb is an important herbal medicinal plant and known due to its antimicrobial, hepatoprotective, immunostimulant, antigenotoxic, antioxidant, antifungal, antihypertensive and anti-inflammatory action (Mushtaq *et al.*, 2016). It has been reported that *Rhubarb* contains different phytochemicals isoazadirolide, nimbandiol, nimbaflavone, nimbinene, quercetin, nimbolide, quercitrin, vilasanin and rutin (Serhat and Muzaffer, 2016). *Coptis chinensis*, is another herbal medicinal plant having antifungal, antibacterial, antiviral, anti-amoeba,

anti-inflammatory, anti-diarrhea, and antioxidant effects (You-ping and Herman, 1995). *Coptis chinensis* is abundant in phytochemicals like mucilage and resins. Another Chinese herb *Codonopsis* could be used as growth promoter in broiler diet. *Codonopsis* is as non-conventional vegetable protein in poultry rations. It is also known as for its antibiotics, hypocholesterolaemic, antiulcerative, anti-inflammatory and diuretic agent (Chen et al., 2012).

Based on therapeutic potentials of *Rhubarb*, *Coptis chinensis*, and *Codonopsis*, we hypothesized that *Rhubarb*, *Coptis chinensis*, and *Codonopsis* could be good herbal growth promoters. Therefore, a study was executed to examine *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract effects on growth performance, blood chemistry, carcass characteristics and digestibility of nutrient in broilers chicks.

Materials and Methods

Current experimental trial was executed on research center of College of Agriculture, Department of Veterinary Science, Ningxia University, China. All procedures and experiments protocol were approved by the Ethic Committee of Animal Experiments, Department of Veterinary Science, College of Agriculture, Ningxia University. All birds under the current trail were handled humanly and no stress was given throughout the experiment. During the experiment animal were given free access to water and commercial to avoid thrust and hunger stress. Birds welfare protocol was followed strictly. In current experiment a total of 300 days old broiler chicks were procured from the local hatchery. Procured day old chicks were housed in floor pens and raised on commercial feed for 7 days to adopt house conditions. After seven days, all chicks were divided into five groups in such a way that each group contained 6 replicates and each replicate had 10 birds (approx.1.2 bird/ft²). The trail was executed in environmental control conditions and light schedule was 21-h light period with 3-h dark cycle. Each replicate was placed in a specific floor pen of dimension 4×3 ft² (Details of birds per replicate are given above). Saw dust was used as a litter material and the depth of litter material was 5–6 cm. Two types of commercial feed, granules and pellet, were used in the current experiment. Granules form of feed was used from day 1 to 21 day of life, while pellet form of feed was used from 22 to 35 days. Six treatment was applied in current experiment in such a way that each treatment had commercial feed *ad libitum* and drinking water with or without supplement. In control treatment drinking water was without any additives; In second treatment drinking water was supplemented with antibiotic (Flavomycin 10mL/liter). The remaining three treatments were supplemented with *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract @ 20 mL/liter, 15 mL/liter, and 10 mL/liter in drinking water, respectively. The brooding temperature was 95°F for first week and then reduces every week 5°F. Local vaccintain schedule was followed. The house relative humidity was maintained between 50 to 70% throughout the trail.

Extraction of Herbs

Rhubarb, *Codonopsis* and *Coptis chinensis* leaves were purchased from College of Agriculture, Department of Botany, Ningxia University, China. Procured leaves were dried at room temperature. Dried leaves were sliced and grinded in hammer mill of sieve size 0.5 mm to obtain fine powder. Fine powder of each Chinese herb i-e *Rhubarb* (4 g), *Codonopsis* (6 g) and *Coptis chinensis* (2 g) was added in 100 mL of purified water. The pH of obtained solutions was maintained at 7 and heated at 80°C for 3 h. The prepared solutions were filtered with ordinary filter paper and cool down for two days at room temperature as described by Farhat et al. (2011).

Performance and Carcass Parameters

Weights of broilers chicks were measured on weekly basis. For this purpose, broiler chicks' final body weight was subtracted from initial body weight every week. Feed consumption data was calculated on daily basis throughout the trail period. For carcass parameters, at day 35 of trial, four birds per replicate were randomly selected, weighed and slaughtered by using a conventional neck cut method to cut the carotid artery and jugular vein, and bled for two minutes. Carcasses were divided to measure the weights of heart, liver and gizzard as well as dressed weight, live weight, thigh weight, breast weight and the abdominal fat weight.

Nutrient Digestibility

At the day 28-29 of the experiment, feces and feed were collected and weighted by 12 birds from each experimental group (2birds/replicate). For this purpose, each bird was kept separately in individual metabolic pens with feces collecting trays under each pen. Feces were collected for forty-eight hours to measure nutrient digestibility according to Leeson and Summers. Then, the collected samples were placed in forced air oven at 60°C for 24 h to dry samples. After drying of samples, all samples were thoroughly homogenized. Representative samples were taken and ground by small hammer mill for further lab analysis. Proximate analysis of ground samples was done according to recommended procedure of AOAC with some modification as followed by Rahman et al. (2019).

Blood Biochemistry and Immune Performance

At last day of the experiment, blood samples (5 mL) were also collected from slaughtered birds. The serum (used for further analysis) was obtained from four blood samples from each replicate in sanitized serum tubes and stored (-20°C) until used for the measurement of blood cholesterol and glucose by using standard kit (Biomega) procedure via biochemistry analyzer (Techno-786). Similarly, blood samples from each replicate were also placed in EDTA tubes

for hemoglobin concentration (Hb) and packed cell volume (PCV) analysis. Hb and PCV were determined using Blood Chemistry Analyzer (Sysmax KX-21). Compound microscope was used to obtain differential leukocyte count of blood samples.

For measurement of immune response, 6 birds were randomly selected from each treatment group at day 7, 14, 21 and 28 of trail. Birds are weighed in morning and dissected to collect the thymus, spleen and bursa of fabricius for organ weight. following formula was used to check immune organ index:

Immune organ index (mg/g) = immune organ weight/live weight

Statistical Analysis

All collected data were analyzed by using standard Analysis of Variance Technique (ANOVA) and Completely Randomized Design (CRD) was employed. Least significance difference was used to compare treatment means differences.

Results

The effect of *Rhubarb*, *Coptis chinensis* and *Codonopsis* extract supplementation in drinking water on performance and carcass parameters of growing broiler were summarized in Table 1. Increased in body weight and breast meat yield had been observed in broilers received drinking water with supplementation of Chinese herbs. Similarly, feed efficiency ratio was improved in broiler received drinking water with supplementation of Chinese herbs ($P < 0.05$). Results revealed that *Rhubarb* extract supplementation in drinking water enhanced the dressing percentage and breast meat yield as compared to control, Flavomycin, *Coptis Chinensis*, and *Codonopsis* ($P < 0.05$). However, liver weight, thigh weight, heart weight and abdominal weight was not influenced by dietary treatment ($P > 0.05$). Moreover, no differences were observed on feed intake, dressing percentage and breast weight between control group and chickens received drinking water with supplementation of antibiotics ($P > 0.05$).

Table 2 represent the nutrient digestibility of broiler on Chinese herbs supplementation in drinking water. Supplementation of flavomycin, *Rhubarb*, *Coptis Chinensis*, and *Codonopsis* in drinking water increased CP and EE digestibility compared with control ($P < 0.05$). However, no significant differences were found in broilers received flavomycin and Chinese herbs extract supplementation in drinking water. Crude fiber digestibility was not affected by supplementation of flavomycin and Chinese herbs in drinking water.

Blood chemistry statistical analysis revealed that addition of Chinese herbal extract into drinking water showed a significant effect on blood cholesterol, glucose and red blood cells (RBC) ($P < 0.05$) (Table 3). Supplementation of flavomycin, *Rhubarb*, *Coptis Chinensis*, and *Codonopsis* in

drinking water resulted in a significant decrease in cholesterol and increase in RBC compared with control ($P < 0.05$). The study finding showed a significant ($P < 0.05$) reduction of blood glucose in the broilers received drinking water with supplementation of *Rhubarb*. However, white blood cells WBC, hemoglobin, and PCV stayed same due to the supplementation of Chinese herbal extracts, and antibiotics as compare to control.

Results of immune organ weight and immune index are presented in Table 4 and 5, respectively. Supplementation of Chinese herbs in drinking water enhanced the weight of thymus and spleen at week 1, 2, 3 and 4 ($P < 0.05$). Highest bursa weight at week 4 was observed in birds supplemented *Rhubarb* in drinking water ($P < 0.05$). Results of immune organ index revealed that *Rhubarb* and *Codonopsis* significantly influence thymus, spleen and bursa immune index at week 1 ($P < 0.05$). At week 2 highest immune organ index for bursa and thymus was observed for *Codonopsis*, while for spleen was observed in *Coptis Chinensis*. Highest immune organ index for spleen, thymus and bursa was observed in *Coptis Chinensis*, *Rhubarb* and *Codonopsis* at week 3, respectively ($P < 0.05$). Interestingly, at week 4 highest immune organ index for thymus, and spleen was observed in control group, however, highest immune organ index for bursa was observed in *Rhubarb* treatment.

Discussion

Results of the current study showed that supplementation of Chinese herbs increased body weight gain, improved feed efficiency and breast muscle weight. Increased body weight gain, improved feed efficiency and breast muscle weight might be attributed to the growth promoting effect of some ingredients of Chinese herbs. Saleh *et al.* (2018) reported that ingredients within herbal extract enhanced the body weight gain, improved efficiency of feed and breast muscle yeild. Current findings of growth are also in consistent with the study of (AL-Kassie 2009; Seyed *et al.*, 2013; Sajid *et al.*, 2015; Omar *et al.*, 2016) who reported enhanced growth performance of birds provided drinking water with *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts. However, contrary results of herbal extract supplementation are also observed in the study of (Wanker *et al.*, 2009) who observed no effect on growth performance of broilers when herbal powder was supplemented in the diet of broiler. The contradiction in literature could be explained by supplementation method i-e trough drinking water or feed. Improved growth performance and better carcass results could be explained by better digestibility of CF and EE in current study. Improved digestibility of CP and EE, while no influence on CF digestibility has been observed in the current study. Current findings are in line with findings of Saleh *et al.* (2018) who observed improved CP and EE digestibility and no effect on CF digestibility in broiler fed herbal extracts.

Blood chemistry results raveled decrease in blood sugar due to supplementation of Chinese herbs extract.

Table 1: Effect of *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts supplementation on growth performance and carcass characteristics of broilers

| | Treatments ¹ | | | | |
|-------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| | Control | Antibiotic | Chinese herbs extract | | |
| | No additive | Flavomycin | <i>Rhubarb</i> | <i>Coptis Chinensis</i> | <i>Codonopsis</i> |
| First body weight (g) | 189±2.240 | 188±1.982 | 185±2.650 | 186±1.982 | 187±1.541 |
| Last body weight (g) | 1837±1.433 ^b | 1930±1.234 ^{ab} | 2034±1.302 ^a | 1972±1.541 ^a | 1951±1.342 ^{ab} |
| Weight gain (g) | 1645±1.302 ^b | 1750±1.302 ^a | 1869±1.241 ^a | 1795±1.302 ^a | 1775±1.432 ^a |
| Feed conversion ratio | 1.85±0.302 ^b | 1.79±0.241 ^a | 1.74±0.302 ^a | 1.78±0.214 ^a | 1.75±0.402 ^a |
| Feed intake (g) | 3394±2.542 ^b | 3440±1.345 ^b | 3578±1.922 ^a | 3475±2.023 ^{ab} | 3415±1.294 ^b |
| Dressing (%) | 64.79±1.422 ^b | 65.25±1.121 ^b | 68.76±1.302 ^a | 64.94±2.982 ^b | 67.15±1.872 ^{ab} |
| Liver (g/100 g body weight) | 2.39±0.982 | 2.51±0.914 | 2.97±0.321 | 2.83±0.092 | 2.42±0.076 |
| Thigh (g/100 g body weight) | 20.20±0.872 | 21.10±0.822 | 22.17±0.098 | 19.34±0.672 | 20.37±0.0762 |
| Breast (g/100 g body weight) | 21.74±0.376 ^b | 21.90±0.641 ^b | 25.42±0.205 ^a | 21.88±0.639 ^b | 22.48±0.302 ^b |
| Heart (g/100 g body weight) | 0.45±0.153 | 0.55±0.125 | 0.56±0.109 | 0.57±0.012 | 0.58±0.092 |
| Abdominal fat (g/100 g body weight) | 3.17±0.612 | 3.13±0.642 | 3.14±0.088 | 3.09±0.201 | 2.92±0.164 |
| Gizzard (g/100 g body weight) | 1.40±0.232 ^b | 1.58±0.302 ^a | 1.64±0.098 ^a | 1.66±0.225 ^a | 1.46±0.032 ^b |

Means with different superscripts shows significant difference ($P < 0.05$). ¹Treatments: control (no supplementation), antibiotics (Flavomycin 10ml/liter), Chinese herb extract, *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract @ 20 mL/liter, 15 mL/liter, and 10 mL/liter in drinking water

Table 2: Effect of *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts supplementation on digestibility of nutrients

| | Treatments | | | | |
|---------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| | Control | Antibiotic | Chinese herbs extract | | |
| | No additive | Flavomycin | <i>Rhubarb</i> | <i>Coptis Chinensis</i> | <i>Codonopsis</i> |
| Ether extract | 84.25±1.201 ^b | 86.58±0.912 ^a | 87.44±1.353 ^a | 86.89±0.781 ^a | 87.00±0.281 ^a |
| Crude fiber | 12.81±1.302 | 16.10±0.907 | 17.92±0.955 | 14.85±0.982 | 15.25±0.811 |
| Crude protein | 71.99 ±0.403 ^b | 73.11±1.560 ^a | 74.96±0.921 ^a | 72.89±0.541 ^{ab} | 73.25±0.671 ^a |

Means with different superscripts shows significant difference ($P < 0.05$). ¹Treatments: control (no supplementation), antibiotics (Flavomycin 10ml/liter), Chinese herb extract, *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract @ 20 mL/liter, 15 mL/liter, and 10 mL/liter in drinking water

Table 3: Effect of *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts supplementation on broilers blood chemistry

| | Treatments | | | | |
|----------------------|---------------------------|----------------------------|---------------------------|---------------------------|----------------------------|
| | Control | Antibiotic | Chinese herbs extract | | |
| | No additive | Flavomycin | <i>Rhubarb</i> | <i>Coptis Chinensis</i> | <i>Codonopsis</i> |
| Packed cell volume % | 26.86±0.509 | 27.97±8.380 | 29.71±8.380 | 27.66±1.380 | 30.00±8.380 |
| Cholesterol (mg/dl) | 134.00±0.143 ^a | 128.58±0.096 ^b | 124.67±0.921 ^b | 123.00±0.806 ^b | 124.00±1.830 ^b |
| Hemoglobin (g/dl) | 9.56±0.306 | 9.80±0.750 | 10.21±0.153 | 9.69±1.030 | 10.38±0.409 |
| White blood cell % | 204.51±0.621 | 210.23±0.306 | 235.00±1.830 | 206.00±1.409 | 222.00±0.123 |
| Red blood cell % | 2.10±0.104 ^b | 2.17±1.830 ^a | 2.28±1.109 ^a | 2.13±1.091 ^a | 2.21±1.019 ^a |
| Blood sugar (mg/dl) | 208.85±1.130 ^a | 204.57±0.104 ^{ab} | 191.23±0.243 ^c | 203.83±1.02 ^{ab} | 194.66±0.113 ^{bc} |

Means with different superscripts shows significant difference ($P < 0.05$). ¹Treatments: control (no supplementation), antibiotics (Flavomycin 10ml/liter), Chinese herb extract, *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract @ 20 mL/liter, 15 mL/liter, and 10 mL/liter in drinking water

Findings are in consistent with the study of (Ayorinde *et al.*, 2017; Sajid *et al.* 2015) who examined decrease of blood sugar level in broilers fed herbal extract. The decrease in blood glucose could be explained by suppressive influence of herbals extracts on glucagon (Gong *et al.*, 2014). Cholesterol level in current study are in line with findings of (Seyed *et al.*, 2013) who reported that herbal plant leaves extract as a substitute of antibiotic reduce the cholesterol level of birds. No change of blood hemoglobin was observed in experimental treatments in current study. The current results are also consistent with (Salarya *et al.*, 2014) who reported that addition of herbal plant leaves extract in broilers drinking had no effect on hemoglobin. (Mwale *et al.*, 2014) also reported that that addition of herbal plant leaves extract in broilers drinking had no effect on hemoglobin. In the current study, PCV was also not changed by treatments. It has been reported that addition of herbs leaf extract has no influence on PCV (Saber, 2011). Findings of WBC in

current are in line with results of (Alireza *et al.*, 2012) observed that addition of herbs leaf extracts in the diet of broiler had no influence on WBC.

The results of immune organ weight and immune index are well matched with Sajid *et al.*, 2015 reported that the supplementation of herbs in chicken increased the immunity of chicken. Zhang *et al.* (2013) found that addition of Astragalus herb powder in feed showed significant difference in immune organs weight of broilers. Thymus, spleen and bursa of fabricius are the most important immune organs which have vital role for immunity in poultry (Qamar *et al.*, 2019). Supplementation of Chinese herbs in drinking water enhanced the weight of thymus, spleen and bursa weight which represent better immunity in birds and can be well defended by better growth performance (Zhang *et al.*, 2013). Results of immune organ index revealed that *Rhubarb*, *Codonopsis* and *Coptis Chinensis* significantly influence thymus, spleen and bursa

Table 4: Effect of *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts supplementation on weight (g) of immune organs in broilers

| | Organs | Treatments | | | | |
|--------|--------|----------------------------|--------------------------|---------------------------|---------------------------|----------------------------|
| | | Control | Antibiotic | Chinese herbs extract | | |
| | | No additive | Flavomycin | <i>Rhubarb</i> | <i>Coptis Chinensis</i> | <i>Codonopsis</i> |
| Week 1 | Thymus | 0.213±0.098 ^c | 0.277±0.156 ^a | 0.266±0.093 ^a | 0.257±0.106 ^{ab} | 0.259±0.146 ^a |
| | Spleen | 0.107±0.038 ^c | 0.137±0.089 ^b | 0.178±0.085 ^a | 0.135±0.099 ^{bc} | 0.140±0.079 ^{ab} |
| | Bursa | 0.150±0.075 ^{bc} | 0.167±0.056 ^b | 0.195±0.096 ^a | 0.157±0.086 ^b | 0.173±0.046 ^{abc} |
| Week 2 | Thymus | 0.602±0.213 ^{ab} | 0.611±0.227 ^b | 0.781±0.197 ^a | 0.605±0.207 ^a | 0.641±0.267 ^a |
| | Spleen | 0.246±0.101 ^c | 0.241±0.068 ^b | 0.209±0.041 ^{ab} | 0.242±0.098 ^a | 0.239±0.078 ^{ab} |
| | Bursa | 0.432±0.088 ^c | 0.470±0.191 ^b | 0.530±0.133 ^a | 0.460±0.111 ^b | 0.480±0.811 ^a |
| Week 3 | Thymus | 1.047±0.375 ^{abc} | 1.120±0.262 ^a | 1.013±0.283 ^{ab} | 1.110±0.212 ^a | 1.110±0.162 ^a |
| | Spleen | 0.436±0.121 ^c | 0.395±0.143 ^b | 0.621±0.233 ^a | 0.385±0.153 ^b | 0.435±0.243 ^b |
| | Bursa | 0.647±0.291 ^{bc} | 0.798±0.309 ^a | 0.625±0.321 ^a | 0.795±0.319 ^b | 0.791±0.409 ^{ab} |
| Week 4 | Thymus | 1.945±0.831 ^c | 2.156±0.695 ^a | 2.279±0.319 ^a | 2.146±0.615 ^a | 2.157±0.795 ^{ab} |
| | Spleen | 0.655±0.191 ^c | 0.862±0.292 ^a | 0.718±0.161 ^{ab} | 0.852±0.272 ^b | 0.868±0.292 ^a |
| | Bursa | 0.316±0.123 ^{bc} | 0.386±0.182 ^b | 0.457±0.111 ^a | 0.366±0.102 ^{bc} | 0.389±0.382 ^{bc} |

Means with different superscripts shows significant difference ($P < 0.05$). ^aTreatments: control (no supplementation), antibiotics (Flavomycin 10ml/liter), Chinese herb extract, *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract @ 20 mL/liter, 15 mL/liter, and 10 mL/liter in drinking water

Table 5: Effect of *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts supplementation on immune organ index (mg/g) in broilers

| | Organ | Treatments | | | | |
|--------|--------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | Control | Antibiotics | Chinese herbs extract | | |
| | | No additive | Flavomycin | <i>Rhubarb</i> | <i>Coptis Chinensis</i> | <i>Codonopsis</i> |
| Week 1 | Thymus | 0.264±0.089 ^c | 0.331±0.211 ^a | 0.317±0.106 ^a | 0.311±0.201 ^b | 0.312±0.221 ^a |
| | Spleen | 0.146±0.077 ^{bc} | 0.161±0.109 ^b | 0.213±0.104 ^a | 0.151±0.119 ^{bc} | 0.160±0.105 ^a |
| | Bursa | 0.209±0.135 ^{ab} | 0.195±0.062 ^b | 0.227±0.092 ^a | 0.185±0.073 ^c | 0.192±0.053 ^{ab} |
| Week 2 | Thymus | 0.414±0.153 ^{ab} | 0.391±0.103 ^{bc} | 0.435±0.095 ^{ab} | 0.394±0.123 ^{ab} | 0.389±0.112 ^a |
| | Spleen | 0.167±0.061 ^a | 0.157±0.046 ^{ab} | 0.116±0.025 ^{bc} | 0.147±0.035 ^a | 0.154±0.054 ^{ab} |
| | Bursa | 0.281±0.059 ^{ab} | 0.287±0.095 ^b | 0.288±0.056 ^{ab} | 0.282±0.084 ^{ab} | 0.284±0.085 ^a |
| Week 3 | Thymus | 0.344±0.132 ^c | 0.405±0.065 ^a | 0.395±0.082 ^b | 0.415±0.075 ^a | 0.397±0.076 ^a |
| | Spleen | 0.146±0.060 ^{bc} | 0.157±0.053 ^b | 0.219±0.074 ^a | 0.147±0.042 ^b | 0.151±0.064 ^b |
| | Bursa | 0.215±0.097 ^b | 0.293±0.125 ^a | 0.218±0.074 ^a | 0.293±0.125 ^b | 0.289±0.134 ^a |
| Week 4 | Thymus | 0.452±0.162 ^a | 0.482±0.105 ^a | 0.513±0.084 ^a | 0.472±0.124 ^a | 0.480±0.124 ^a |
| | Spleen | 0.172±0.063 ^a | 0.211±0.068 ^b | 0.159±0.045 ^b | 0.201±0.015 ^{ab} | 0.204±0.057 ^{bc} |
| | Bursa | 0.078±0.031 ^c | 0.091±0.042 ^b | 0.099±0.026 ^a | 0.081±0.032 ^a | 0.090±0.051 ^{ab} |

Means with different superscripts shows significant difference ($P < 0.05$). ^aTreatments: control (no supplementation), antibiotics (Flavomycin 10ml/liter), Chinese herb extract, *Rhubarb*, *Coptis chinensis*, and *Codonopsis* extract @ 20 mL/liter, 15 mL/liter, and 10 mL/liter in drinking water

immune index at different weeks representing better immune status (could be seen in supplementary Table 1) of birds supplemented with herbs extract.

Conclusion

Rhubarb, *Coptis chinensis* and *Codonopsis* leaf extracts supplementation in drinking water improve growth performance, feed conversion ratio, dressing percentage, gizzard weight, breast meat yield, crude protein and ether extract digestibility. *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts supplementation in drinking water also improve blood chemistry parameters, immune organ weight and immune indexes. Therefore, *Rhubarb*, *Coptis chinensis* and *Codonopsis* leaf extracts has potential to be alternative of antibiotic (flavomycin) growth promotor.

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